Retrospect

THE MECHANISM OF PIGMENT FORMATION IN THE SKIN.*

A Review

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An autochthonous pigment, melanin, is normally present in the cytoplasm of the cells of the basal layer of the epidermis, the germinal layer of the hair bulbs, and the cells of the hair matrix, and where there is marked pigmentation, pigment granules may also occur in the polyhedral cells of the prickle cell layer. This pigment, melanin, was originally considered to be of epidermal origin, for Erasmus Wilson[1] in his textbook in 1852 says: "There is another feature in the history of the development of the epidermal cell, which I find peculiarly interesting. This relates to an organic change taking place in the assimilative powers of the primitive granules, by which the latter are altered in their colour, in short, are converted into pigment granules. Pigment granules appear to differ in no respect from the primitive granules, excepting in tint of colour and chemical composition." Pigment is also found in certain cells in the upper layers of the cutis, which are scattered here and there among the collagen fibres. Kolliker[2] in 1860, first put forward the view that melanin was formed in these cells in the upper layers of the cutis, and from there was transported to the epidermis. Ehrmann[3] also supported this view, believing that melanin was formed only in these melanoblasts of the cutis, and that they acted as wandering cells and transported the pigment to the cells of the basal layer of the epidermis. The upholders of the epidermal origin of melanin looked on these pigment-bearing cells of the cutis as cells which were not able to produce pigment, but rather that they carried away the excess of pigment from the epidermis.

Meirowsky[4] demonstrated beyond any question of doubt that melanin is formed by epidermal cells, but the origin of the pigment found in the pigment-bearing cells of the cutis is still a subject for argument and surmise.

It is due to the researches of Bloch[5] that further light has been turned on the formation of melanin. The knowledge in regard to details of the formation of certain pigments in the plant kingdom led him to believe that the same process might be responsible for the production of melanin. Schonbein showed that potatoes, certain poisonous fungi, wheat gluten and red blood cells have the ability to activate the oxygen of the air, and as a result of the oxidation process, certain coloured products are formed. Yoshidas in 1883, determined the presence of a specific ferment, laccase, in the milky fluid of the lacquer tree, which caused the black colour when the sap of the tree was exposed to air. Then again, certain animal oxydases were known—tyrosin-oxydase or tyrosinase, which is widely present in the animal kingdom, activates tyrosin, phenol and certain near related peptone combinations. This ferment has been shown to be present in the intestines of mealworms, in the hæmolymph of butterfly wings, and at one time was thought to explain pigmentary processes of the skin. Further, polyphenolase, a ferment, is widely distributed in the animal and plant kingdom and activates polyphenols and animo-acid combinations. Many reagents may be used to determine the presence of this ferment, among others such substances as guaiacol, phenolphthalein and adrenalin. The reaction best suited for its determination is the Rohman-Spitzer[6] method. This depends upon the oxydation of paraphenylendiamine by the ferment and the union of the resulting product with a-napthol giving a blue reaction, the so-called indol-phenol blue reaction of Ehrlich. Phenolase is considered to be the cause of the colour changes which occur when apples, pears, potatoes and the juice of certain poisonous fungi are exposed to the air. This ferment has further been demonstrated in the crushed pulp of certain organs, and Winkler showed its presence in the granules of leucocytes of myelogenous origin, obtained in pus or blood.

Bloch first applied the indol-blue method to fresh skin sections, but the details of the reaction were not very clear, and permanent mounts were difficult because the reaction product was soluble in zylol and alcohol. He therefore devised a special method to obviate this difficulty. This was to put fresh skin sections in 2 — 3% solution

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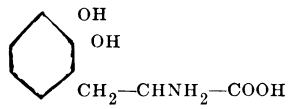
of agar in distilled water. After cooling, a block was cut out, which was frozen on the freezing microtome, and sections, fifteen microns in thickness, were cut at an acute angle. These were then overrun with a 1% a-napthol and paraphenylendiamine and the reaction observed microscopically. The results of the application of these reagents was as follows: There was no reaction in the epidermis, follicular epithelium or hairs. In the cutis the reaction was positive in the cytoplasm of the leucocytes. The active secreting part of the sweat glands gave the blue reaction, while the epithelium of the conducting tubules was only very slightly positive or negative. The reaction also occurred in the sweat of individuals, and this reaction was destroyed by boiling. In pathological skin sections the myelogenous elements gave a *very definitely positive reaction*, whereas those of lymphatic origin were quite negative.

Dyson[7] held the view that pigment in the epidermis resulted from nuclear activity, and was a lipochrome in origin. Bloch, as a result of his experimental work, held that pigment production in the skin is the result of the action of a specific ferment, and is intimately bound up with an oxydation process. He first treated freshly cut frozen sections with certain di-and tri-oxyphenols, pyrocatechin and other substances, with no very good results. He then used a substance dioxyphenylalanin—for short termed "dopa."—This substance is a combination product of pyrocatechin with a-amino proprionic acid and has the formula



There are two varieties of this substance, a natural variety occurring in the germinal centres of vicia faba, which is optically active, and a synthetically prepared variety. While the natural variety yielded the best results, the synthetically prepared one was found satisfactory. Frozen agar sections were placed in a watch glass containing .1 — .2% aqueous "dopa," the watch glass was guarded against evaporation and kept at room temperature for twenty-four hours or in a thermostat for eight or more hours, depending on the degree of reaction. The sections were then washed in distilled water, transferred to a slide, counterstained and mounted in Canada Balsam.

With a slightly positive reaction the reacting cells were filled with a smoky gray to grayish-black coloured material. Where a stronger reaction resulted, the cells were filled with brownish, black, round or angular granules which were deposited only in the cytoplasm of the cell, the nucleus remaining clear or taking the counter stain. In the epidermis, the basal layer cell, the epithelial germinal layer of the hair bulb and the cells of the hair matrix gave a positive reaction. Where excessive reaction occurred the whole Malphigian layer might take part. In these reacting cells the reaction was identical in disposition with the true melanin granules. The reaction took place in two varieties of cells: (1) the normal, cylindrically shaped cells of the epidermal basal layer, (2) dendritic shaped cells or cells of Langerhans, which have many or a few prolongations extending into the interspaces, scattered throughout the basal layer, and these latter cells, particularly the processes, were richly strewn with granules. Bloch found that these cells were much more numerous in sections treated with "dopa" than had been previously thought, as the prolongations of these cells do not stain with ordinary basic or acid dyes, although the cell body does take these stains. Reducing agents, such as silver nitrate, do stain these processes. Bloch found that these Langerhans cells were especially numerous in hyperpigmentary processes, and he considered them to be, morphologically, specialized epidermal cells. In the cutis certain elements alone react. The secreting cells of the sweat glands give the reaction and the sweat also to a moderate degree. Cells of myelogenous origin reacted strongly, whereas those of lymphatic origin were completely negative. In short, the reaction in the cutis paralleled the results obtained by the phenol indol blue method.

Now, whereas phenolase apparently activates a variety of substances the ferment in the epidermal cells on the other hand, is apparently specific, as no other substances even closely related in their chemical structure to "dopa", have been found to demonstrate its presence.

That this "dopa" reaction is essentially an oxidative process is evidenced (1) By the formation of a black sediment when "dopa" was exposed to the air in vitro, (2) Under anaerobic conditions the results were completely negative.

In normal healthy skin this reaction varied. It is noteworthy that "dopa" melanin was deposited at the sites of true melanin granules Usually in hyperpigmented skin, the reaction

was marked, in fair skins, it was weakly positive. That this reaction is intimately bound up with pigment production is not to be doubted, for in spotted animals the non-pigmented or albinotic areas were completely negative, whereas the pigmented areas reacted strongly. Then again, in vitiligo, the leucodermatous areas were completely negative, the hyperpigmented zone reacted strongly, the reaction gradually fading in intensity towards the normal skin. This shows that vitiligo is the result of a local loss of pigment ferment, and is not apparently dependent on any general cause, this being quite in keeping with our clinical knowledge. It sometimes occurred that the reaction was weak in the presence of an obvious hyperpigmentation, but this might be explained by the view that hyperpigmentation is the end result of a fermentative energy that has become weak and inactive.

In pathological conditions the "dopa" reaction also supports the view that "dopa" ferment is intimately connected with pigment production. In Addison's disease, the reaction was not excessive; it was practically normal. Therefore it is urged by Bloch that the increased pigmentation in Addison's disease cannot be due to the presence of an increase of ferment, but rather must be the result of an increased amount of melanin mother substance in the blood or lymph stream. He considers that melanin and adrenalin probably arise from the same or closely allied substances, and that the inability of the suprarenal gland to produce adrenalin results in an increased amount of this precursor substance in the blood stream, that possibly this substance is of a toxic nature and that the hyperpigmentation in Addison's disease may therefore be explained as a protective process on the part of the body. But as Kyrle[8] points out, the sympathetic system must surely play a rôle in the control of pigment production, for there are cases, as for example, where pigmentation and other symptoms may develop very similar to those occurring in Addison's disease, which are due to disturbances of the proper function of the sympathetic ganglia by tumours of the retro-peritoneal region. Anatomically the suprarenal medulla may be quite intact. Lesions which affect the sympathetic must also react on the medulla of the suprarenal gland, it being a modified neurogenous tissue, and equally so, lesions of the medulla of the suprarenal gland must surely cause a disturbance of the sympathetic nervous control. Just how the sympathetic nervous system controls or is related to the pigment function of the skin cannot as yet be more fully explained.

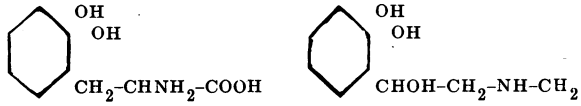
Radiant energy caused an increased activity of the ferment, and in local hyperpigmentary processes as in ephilides and chloasma there was a strong reaction to "dopa". Further, pigmented nævi reacted strongly to "dopa," as might be expected in view of their epiblastic origin.

The pigment bearing cells of the cutis did not give any reaction to "dopa" ferment. The pigment in these cells is as far as is known melanin, and these cells are usually found to lie under an area of the epidermis especially rich in pigment. These facts have lent support to the view that these cells have not the ability to originate pigment, but are able to transport excess of pigment from the epidermis, *i.e.*, that they are chromatophoric and not melanoblastic in function. It may be that the ultimate fate of melanin, which is not now known, may well be bound up with the presence of these cells.

The view that the formation of melanin is a function of epidermal tissue, and is not primarily of mesodermal origin is that most widely held today. There is, however, a notable exception to which reference must be made. In the new born there may be observed—rarely, it is true—practically exclusively in the sacral region, certain discrete pigmented macules. These are designated "Mongolian spots" and tend to gradually disappear as the child becomes older. They are due to certain cells of characteristic shape and disposition. These cells are elongated, fibrillary and often have pseudopodia-like processes, and are scattered irregularly throughout the deeper layers of the cutis. It has been shown that similar cells are to be found in the lower animals, particularly apes, where they have apparently a definite function, and therefore in the human, are to be considered as survivals of cells that, phylogenetically, are doomed to disappear. Such cells are only of importance under pathological conditions but it is not to be doubted that they produce their own pigment.

To sum up, the present status of our knowledge of the pigment production of the skin is that pigment is produced in the epidermis by means of a specific ferment in the cytoplasm of the cells of the germinal layer, and that this ferment activates a specific substance brought to the cells by the blood or lymph stream, and further that this process is essentially an oxidative process. Melanin is a highly complex, highly stable substance containing sulphur. It

may be that in its structure it is related to "dopa," but this is as yet undetermined. Adrenalin is a combination product of pyrocatechin and is closely related to "dopa," as the formulas show.



Bloch believes that "dopa" occurs in the intermediate metabolism of the body, but this substance has never been shown to occur as a normal metabolic product. On the other hand, the possibility of adrenalin, tyrosin, tryptophan and homogenistic acids being a mother substance of melanin would appear to be definitely negatived.

REFERENCES

(1) ERASMUS, WILSON, *Diseases of the Skin*, 1852. (2) KOLLIKER, Woher stammt das Pigment in der Oberhaut gebildet, *Anat. Anzeiger*, 1887. (3) EHRMANN, S., Zur Kenntnis von des Pigmentes bei den Amphibien, *Archiv. für Dermat. ü. Syphilis*, 1885-1886, 12/13, 509. (4) MEIROWSKY, Uber den Ursprung des Melanotischen Pigmentes der Haut und des Auges, Leipzig, 1908. (5) BLOCK, BR. UND RYHINER, P., Histachemische Studien in überlebenden Geürbes über fermentative Oxydation und Pigment Bildung, *Zeitschrift für die gesamte experimentelle Medizin*, 1917. (6) ROHMANN-SPITZER, Uber Oxydationswirkungen tierischer Gewebe, *Berichte der Deutsch. Ghem. Gesellschaft*, 1895, xxviii, 587. (7) DYSON, W., Cutaneous Pigmentation, *Brit. Jour. of Derm. & Syph.*, 1911, xxiii, 205. (8) KYRLE, J., *Histobiologie der Menschlichen Haut*, 1925.

RECENT ADVANCES IN HÆMATOLOGY

1. THE ORIGIN OF THE RED BLOOD CELL IN ADULT MARROW

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In adult mammalian and avian bone marrow the progenitor of the red blood cell arises from the endothelium of the bone marrow capillaries and the process of maturation takes place within these endothelial lined channels. Such is the conclusion of Dr. Florence Sabin and her coworkers, after a comprehensive study of the bone marrow of rabbits and pigeons.

Experimental Methods. In order to simplify the study, the marrow was first reduced to an extremely hypoplastic state. Observations during this stage and after the marrow had been permitted to regenerate itself, were made from material obtained by trephining the bone on the living animal or after sacrificing the animal. The experimental means adopted were simple and effective. In pigeons hypoplasia was obtained by starving. Regeneration was prompt after feeding. The marrow of rabbits was simplified by the depletion of its myeloid elements by several injections of inactivated typhoid bacilli given at forty-eight hour intervals. Hyperplasia was effected by bleeding the animals. The vascular system was studied after the injection of India ink.

Results. In hypoplastic marrows two kinds of intercommunicating capillaries are encountered; the sinuses or sinusoids containing the actively circulating blood, and intersinusoidal capillaries, which are potential cavities with their endothelial walls in apposition. At either end, the walls of these latter channels are directly continuous with the patent sinuses. From the endothelium of these collapsed or hæmatopoietic capillaries, the primitive progenitor of the erythrocyte, called by these authors the *megaloblast*, buds off by mitosis into the potential capillary lumen. This new cell by indirect division gives rise to erythroblasts and these in turn to many normoblasts. During the process of maturation the walls of the capillary blood vessels become pushed further apart. Finally the cells within them, having reached maturity, distend the capillary until it becomes patent to the ends, when a circulation is established with the communicating sinus, and the newly formed cells are washed into the blood stream. The intersinusoidal capillary thus becomes a patent sinusoid. While this process is going on, other sinusoids collapse to form new hæmatopoietic channels. Under normal conditions where the demand for new erythrocytes is not great, there is little activity on the part of the endothelium of the capillaries, because one megaloblast may be the progenitor of many erythrocytes. The daily demand is probably met by proliferation of the erythroblasts inside the hæmatopoietic capillaries. On the other hand during extreme hyperplasia not only the endothelium of these capillaries becomes active, but in addition, the endothelial cells of the patent sinuses are called into play. This explains why immature cells during this state reach the general circulation, while under ordinary circumstances only the ripe cells are found there.

The erythropoietic and myelogenic areas in the marrow are separate and distinct. The former appear relatively avascular owing to a predominance of the collapsed capillaries, while the latter show more numerous patent sinuses. The granulocytes arise from an extravascular primitive reticular cell; they mature in the marrow